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Microbial BioRemediation – A Review

Kshitija Iyer, Gajalakshmi S., Mythili S. and Sathiavelu A.
School of Biosciences and Technology, Vellore Institute Technology, Vellore, TamilNadu, INDIA
asathiavelu@vit.ac.in

ABSTRACT
The goal in bioremediation is to stimulate microorganisms with nutrients and other chemicals that will enable them to destroy the contaminants. The bioremediation systems in operation today reply on microorganisms native to the contaminated sites, encouraging them to work by supplying them with the optimum levels of nutrients and other chemicals essential for their metabolism. Thus, today's bioremediation systems are limited by the capabilities of the native microbes. However, researchers are currently investigating ways to augment contaminated sites with non-native microbes including genetically engineered microorganisms especially suited to degrade the contaminants of concern at particular sites. It is possible that this process, known as bioaugmentation, could expand the range of possibilities for future bioremediation systems.

Keywords: Wastewater, Bioremediation, heavy metals, microorganisms, biomass, pollution

INTRODUCTION

Heavy-metal pollution in aquatic and soil environments has become a common issue for both developing and developed countries. Heavy metals have been intensively utilized in a variety of industries and are of great environmental concern because of their severe toxicity and unique characteristics. Unlike organic pollutants, heavy metals are non-biodegradable and tend to accumulate and concentrate in living organisms via the food chain [1]. Problems arising from heavy-metal pollution are major threats to human health and to the entire ecosystem. To create a world that can both drive robust industrial growth and to sustain environmental demands, technologies leading to clean-up of heavy-metal pollution are of increasing demand. A range of methods has been used for heavy metal removal, and one of the mostcommonmethods is adsorption. In particular, using natural and environmentally compatible materials (e.g., biomass of microorganisms and plants) for metal adsorption (called biosorption) is of great interest [2 – 4]. Microorganisms and plants with the ability to resist, detoxify, and adsorb metals have been widely studied for their potential to remediate metal-contaminated environments [5, 6].

Water pollution is a major environmental problem faced by modern society that leads to ecological disequilibrium and health hazards. Heavy metal ions such as copper, cadmium, lead, nickel, and chromium, often found in industrial wastewater, present acute toxicity to aquatic and terrestrial life, including humans. Thus, the discharge of effluents into the environment is a chief concern. Many methods, such as chemical precipitation [7], ion exchange [8], membrane processes [9] and adsorption onto activated carbon [10] etc., have been used to remove heavy metal ions from various aqueous solutions. However, the application of such processes is often restricted because of technical or economic constraints [11, 12]. Biosorption of heavy metals is one of the most promising technologies involved in the removal of toxic metal ions from wastewater. It is a potential alternative to conventional processes for the removal of metals due to the low cost, easily obtained, minimization of the volume of chemical and/or biological sludge to be disposed of, high efficiency in detoxifying very dilute effluents and no nutrient requirements [13 - 15]. A great interest has recently been directed to the biosorption of heavy metals from solutions using different biomaterials as adsorbents. Among the various resources in biological wastes, both dead and live biomass, exhibit particularly interesting metal-binding capacities [16, 17]. In recent years, agricultural byproducts have been widely studied for metal removal from water. These include peat, wood, pine bark, banana pith, soybean and cottonseed hulls, peanut, shells, hazelnut shell, rice husk, sawdust, wool, orange peel, and compost and leaves [18].

The introduction of heavy metals, in various forms in the environment, can cause considerable modifications in the structure and function of microbial communities. Heavy metal contamination of soil decreases microbial activity, microbial numbers and microbially mediated soil processes such as nitrification, denitrification and decomposition of organic matter [19 – 23]. Heavy metals can enter ecosystems through a combustion of fossil fuel, use of common antiseptics and disinfectants, exhausted batteries, agricultural practices and as waste of several industrial processes. Heavy metals in low concentration are essential to all living organisms. However, high concentrations of heavy metals in the environment can cause severe toxic effects to exposed plants, animals, and humans [24, 25].

THE BIOLOGICAL AND CHEMICAL PROCESSES OF BIOREMEDIATION

The Biological Process - Microbial bioremediation

Cupriavidus taiwanensis

A novel metal biosorption system consisting of the symbiotic combination of an indigenous metal-resistant rhizobial strain, *Cupriavidus taiwanensis* TJ208, and its host plant *Mimosa pudica* has been developed for the removal of heavy-metal pollutants. Free-living *C.taiwanensis* TJ208 cells were able to absorb 50.1, 19.0,

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and 19.6 mg/g of Pb, Cu, and Cd, respectively. After nodulation via inoculation with strain TJ208, the metal uptake ability of *M. pudica* markedly increased, as the nodulated *M. pudica* displayed a high metal uptake capacity (qmax) of 485, 25, and 43 mg/g, respectively, which is 86, 12, and 70% higher than that of nodule-free plants. Moreover, with TJ208 nodules, the *M. pudica* plant also displayed a 71, 81, and 33% enhancement in metal adsorption efficiency (η) for Pb, Cu, and Cd, respectively. The nodulation appeared to give the greatest enhancing effect on the uptake of Pb, which is consistent with the preference of metal adsorption ability of TJ208. This seems to indicate the crucial role that the rhizobial strain may play in stimulating metal uptake of the nodulated plant. Furthermore, the results show that metal accumulation in the nodulated plant mainly occurred in the roots, accounting for 65–95% of total metal uptake. In contrast, the nodules and the shoots only contributed to 3–12 and 2–23% of total metal uptake, respectively. Nevertheless, the specific adsorption capacity of nodules is comparable to that of the roots. Hence, this work demonstrates the feasibility and effectiveness of using the nodulated plants to promote phyto-removal of heavy metals from the polluted environment as well as to restrict the metal contaminants in the un-harmful region of the plant [1].

Bacillus strains

In this study, bacterial strains were investigated in order to determine their heavy metal tolerance. The bacterial strains were identified as *Bacillus cereus* and *Bacilluspumilus.* In the batch system, the effects of operating variables such as solution pH, initial metal concentration, contact time, and adsorbent dosage were investigated. Both isolates were highly resistance to copper and lead in comparison with the control strain examined. The adsorption capacities of *B. cereus* and *B. pumilus* were found to be 22.1 mg/g and 28.06 mg/g, respectively. The biosorption follows pseudo-second order kinetics and the isotherm fits well to the Langmuir isotherm model. In column experiments, the biosorption was fitted well by the Thomas model. The breakthrough and exhaustion capacity of each biosorbent decreased with increasing flow rate. In the fixed-bed system, the biosorption capacities of, *B. cereus* and *B. pumilus* were observed to be higher than that of the batch system.

In the present study, the *B. pumilus* and *B. cereus* isolated from the same soil were found to be resistant to several heavy metals. Both isolates were highly resistance to copper and manganese in comparison with the other metals examined. The $Pb2^{+}$ uptake capacities of *B. pumilus* and *B. cereus* were investigated in fixed bed column and batch systems. The maximum $Pb2^{+}$ biosorption capacity of the biomasses were obtained at the initial pH of 6.0. The batch experimental results fitted well to Langmuir isotherm model.

The biosorption followed second-order-rate kinetics. Our study findings also indicate that there is no direct relationship between the level of metal resistance and biosorption capacity of the *B. pumilus* and *B. cereus*.[3]

Sphaerotilus natans

The *Sphaerotilus natans* is a Gram-negative bacterium isolated from the waste streams of a waterpurification plant. In the present work a lyophilised cell suspension of *Spherotilus natans* was studied as biosorbent material for cadmium and copper removal from aqueous solutions. The biomass was firstly characterised by potentiometric titration and evaluation of the major ionic content. The experimental data highlight that the biomass cell wall contains two main acidic groups with a total amount of 5 meq. Equilibrium biosorption trials of cadmium and copper were carried out to investigate the effects of two important experimental factors, pH and biomass concentration. As expected, both cadmium and copper biosorption extent was repressed by pH decrease. The effect of the biomass concentration changes both with the equilibrium pH value and the kind of metal adsorbed. In the case of copper biosorption at pH)5, the increase of biomass concentration causes a diminution of the maximum specific metal uptake due probably to cell aggregation phenomena; whereas at acidic pH values the previous trend is inverted perhaps because of the effect of partial hydrolysis of the bacterial cell wall constituents. A different behaviour was observed for cadmium biosorption in relation to the microbial cell concentration: the effect of biomass concentration is less evident and opposite with respect to copper at pH 6 and 3 and no definitive explanation was found for this case. Equilibrium modelling was performed for both metals by using the most used equations reported in the literature. A comparison of the biosorption characteristic of *S. natans* has been also performed with respect to some results reported by other researchers [4].

Geobacillus toebii sub.sp. decanicus and Geobacillus thermoleovorans sub.sp. stromboliensis:

Biosorption of each of the ions Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and $Mn2^{+}$ on *Geobacillus toebii* sub.sp. *decanicus* (G1) and *Geobacillus thermoleovorans* sub.sp. *stromboliensis* (G2) in a batch stirred system was investigated. The equilibrium adsorptive quantity was determined to be a function of the solution pH, contact time, biomass concentration, initial metal concentrations and temperature. The results obtained from biosorption experiments are used to understand the driving forces that govern the interaction between metal ions and a biosorbent. The experimental results were fitted well to the Scatchard plot, Langmuir, Freundlich, Dubinin–Radushkevich (D–R) isotherms. According to the parameters of the Langmuir isotherms, the maximum biosorption capacities of Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and $Mn^{2}+$ for G2 were 38.8, 41.5, 42, 29 and 23.2 mg/g, respectively, while 29.2, 48.5, 21, 21.1 and 13.9 mg/g for G1, respectively. The mean free energy values evaluated from the D–R model indicated that the biosorptions of studied heavy metal ions onto bacteria were taken place by physical interaction. The biosorption mechanisms of studied metal ions on to the biosorbents were analysed by FT-IR spectra of G1 and G2 with and without metal ions. The first order and second order coefficients were obtained at 298, 308, 318 and 343K. The experimental

results were used to calculate the thermodynamic parameters [5].

Enterobacter sp. J1

This study was undertaken to investigate biosorption kinetics and equilibria of lead (Pb), copper (Cu) and cadmium (Cd) ions using the biomassof *Enterobacter* sp. J1 isolated from a local industry wastewater treatment plant. Efficiency of metal ion recovery from metal-loaded biomass to regenerate the biosorbent was also determined. The results show that *Enterobacter* sp. J1 was able to uptake over 50mg of Pb per gram of dry cell, while having equilibrium adsorption capacities of 32.5 and 46.2 mg/g dry cell for Cu and Cd, respectively. In general, Langmuir and Freundlich models were able to describe biosorption isotherm fairly well, except that prediction of Pb adsorption was relatively poor with Langmuir model, suggesting a different mechanism for Pb biosorption. Adjusting the pH value to 3.0 led to nearly complete desorption of Cd from metal-loaded biomass, while over 90% recovery of Pb and Cu ions was obtainedat pH2. After four repeated adsorption/desorption cycles, biomass of*Enterobacter* sp. J1 retained 75, 79 and 90% of original capacity for adsorption of Pb, Cu and Cd, respectively, suggesting good reusability of the biosorbent. A combinative model was proposed to describe the kinetics of heavy-metal adsorption by *Enterobacter* sp. J1 and the model appeared to have an excellent prediction of the experimental data. The model simulation results also seemed to suggest that intracellular accumulation may occur during the uptake of Pb [13].

Arthrobacter sp

A study on the biosorption of toxic metals (copper, manganese, nickel and lead) by a species of *Arthrobacter* is presented. The equilibrium of the process was in all cases well described by the Langmuir isotherm. The highest values of specific uptake were 406 mg Mn/g, 148 mg Cu/g, 13 mg Ni/g and 130 mg Pb/g. Some factorial experiments were performed in order to investigate the effect of operating and cultural conditions on the equilibrium. Experimental results showed the influence of the biomass concentration on the specific uptake (mg of metal/g of biomass as dry weight). A comparison with results reported in the literature was made [14].

Zoogloea ramigera and Rhizopus arrhizus

The biosorption of Fe (III), Cr (VI), Pb (II), Cu (II) and Ni (II) ions on *Zoogloea ramigera* (activated sludge bacterium) and *Rhizopus arrhizus* (filamentous fungus) has been studied as a function of initial metal ion concentration and temperature. The applicability of the Langmuir model for each metal, microorganism system has been tested at different temperatures. The enthalpy change for the biosorption process has been evaluated by using the Langmuir constant b, related to the energy of adsorption. Thermodynamic parameters indicate the exothermic nature of Cu (II) and Ni (II) biosorption on both microorganisms. Fe(III), Cr(VI) and Pb(II) biosorption is determined to be an endothermic process since increased binding occurs as the temperature is increased in the range 15–45°C [15].

Ceratophyllum demersum

Heavy metals can be absorbed by living or non-living biomass. Submerged aquatic plants can be used for the removal of heavy metals. In this paper, lead, zinc, and copper adsorption properties of *Ceratophyllumdemersum* (Coontail or hornwort) were investigated and results were compared with other aquatic submerged plants. Data obtained from the initial adsorption studies indicated that *C. demersum* was capable of removing lead, zinc, and copper from solution. The metal biosorption was fast and equilibrium was attained within 20 min. Data obtained from further batch studies conformed well to the Langmuir Model. Maximum adsorption capacities (qmax) onto *C. demersum* were 6.17 mg/g for Cu (II), 13.98 mg/g for Zn (II) and 44.8 mg/g for Pb (II). Kinetics of adsorption of zinc, lead and copper were analysed and rate constants were derived for each metal. It was found that the overall adsorption process was best described by pseudo second-order kinetics. The results showed that this submerged aquatic plant. *C. demersum* can be successfully used for heavy metal removal under dilute metal concentration [16].

Myriophyllum spicatum

Submerged aquatic plants can be used for the removal of heavy metals. In this paper, the adsorption properties of *Myriophyllum spicatum* (Eurasian watermilfoil) for lead, zinc, and copper were investigated and the results were compared with other aquatic submerged plants. Data obtained from the initial batch adsorption studies have indicated that *M. spicatum* is capable of removing lead, zinc, and copper from solution. Metal biosorption was fast and equilibrium was attained within 20 min. Data obtained from further batch studies fitted the Langmuir model. The maximum adsorption capacities (qmax) were 10.37 mg/g for Cu (II), 15.59 mg/g for Zn (II) and 46.49 mg/g for Pb (II). The kinetics of adsorption of zinc, lead and copper were also analysed and rate constants were derived for each metal. It was found that the overall adsorption process was best described by the pseudo second order kinetics. The results showed that this submerged aquatic plant *M. spicatum* can be successfully used for heavy metal removal [17].

Bacillus sphaericus

The tolerance to As, Hg, Co, Fe and Cr was determined in different Colombian *Bacillus sphaericus* native strains, as well as the biosorption and bioaccumulation in living biomass. In addition, biosorption of Cr in dead cells was also determined. Living cells of the two most tolerant strains had the capacity to accumulate between 6 and 47% of Co, Hg, Fe and As. Living and dead cells of *B.sphaericus* OT4b31 showed a biosorption of 25 and 44.5% of Cr respectively, while *B. sphaericus* IV(4) showed a biosorption of 32 and 45%. These results are due to the absence of an active metabolism in dead cells and to the pH adjustment. S-layer proteins may possibly have the ability to entrap metallic ions, either on living or

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dead cells. This can bean interesting alternative for bioremediation processes of heavy metals [18].

Brown seaweed

The removal of heavy metals by a dry biomass of brown seaweed was evaluated. Acontinuous system was used, with an effluent from a Brazilian zinc-producing industry,containing zinc (88.0mg/L), cadmium (1.4 mg/L), and manganese (11.7mg/L), as wellas high levels of calcium (4447mg/L), magnesium (100 mg/L) and sodium (37.0mg/L).Preliminary results, in batch conditions, indicated fast uptake kinetics for the heavymetals, whose equilibria were reached in a maximum of 30minutes. The continuous runwas conducted in a laboratory acrylic column, lm high, containing several samplers,filled with the dry biomass. The system operated in up flow condition, at a flow rate of 25mL/min, for approximately 70hours, with high operational stability. The results showed high efficiency in the biosorption of heavy metals. Sodium, calcium and magnesium were not incorporated by the biomass, probably as they are present in the structural polysaccharides of the biomass, thus preventing the establishment of an effective ion exchange process. Analysis of the obtained results did not indicate selective uptake of the metals, probably due to their marked concentration differences in solution. The continuous laboratory system initially showed efficiency close to 100% in the biosorption of all heavy metals, followed by a gradual decrease, as a function of the saturation of binding sites in the biomass. A mathematical adjustment of the curves obtained for the uptake of the different metals was used for estimating the amount of biosorbed metals, through mathematical computer integration [20].

Riccia fluitans

In the present study, biosorption and bioaccumulation characteristics of *Riccia xuitans* to be used as biological mineral feed supplement, was investigated. Preliminary studies showed that *R. xuitans* was rich in protein (27–31%) and possessed high cation exchange capacity (14.5meq) and therefore it has the potential to find an application as biological carrier of microelements that are supplied to feed of animals, the diet of which is deficient in these components. In the present study, various processes of enrichment with microelements of crystalwort were investigated, including biosorption, bioaccumulation by non-growing and growing cells in single-(Cr (III) ions) and multi-metal system (Cr (III), Cu (II), Mn (II), Zn (II) ions). The effect of process parameters (temperature and pH) on metal ions binding efficiency was studied in single-metal system. It was found that at 20°C and pH5 the biomass bound 106mg g¡1 Cr (III) ions. The experimental results showed that the mostly advantageous process of metal ions binding was biosorption, the process that is also the mostly cost-effective [21].

Filamentous fungi

Heavy metal analysis of agricultural field soil receiving long-term (>20 years) application of municipal and industrial wastewater showed twoto five-fold accumulation of certain heavy metals as compared to untreated soil. Metal-resistant fungi isolated from waste water treated soil belonged to genera *Aspergillus, Penicillium, Alternaria, Geotrichum, Fusarium, Rhizopus, Monilia* and *Trichoderma*. Minimum inhibitory concentrations (MIC) for Cd, Ni, Cr, Cu, and Co were determined. The MIC ranged from 0.2 to 5 mg $ml^{-1}$for Cd, followed by Ni (0.1–4 mg ml^{-1}), Cr (0.3–7 mg ml^{-1}), Cu (0.6–9 mg ml^{-1}) and for Co (0.1–5 mg ml^{-1}) depending on the isolate. *Aspergillus* and *Rhizopus* isolates were tested for their metal biosorption potential for Cr and Cd in vitro. Biosorption experiments were conducted with initial metal concentrations of 2, 4, 6 and 8 mM with a contact time of 4 h and wet fungal biomass (1–5 g) at 25^{o} C. Maximum biosorption of Cr and Cd ions was found at 6 mM initial metal concentration. *Aspergillus* sp.1 accumulated 1.20 mg of Cr and 2.72 mg of Cd per gram of biomass. Accumulation of these two metals by very tolerant *Aspergillus* sp.2 isolate was at par with relatively less tolerant *Aspergillus* sp.1 isolate. *Rhizopus* sp. accumulated 4.33 mg of Cr and 2.72 mg of Cd per g of biomass. The findings indicated promising biosorption of cadmium and chromium by the *Rhizopus* and *Aspergillus* spp. from aqueous solution. There is little, if any, correlation between metal tolerance and biosorption properties of the test fungi[23].

Metal resistant bacterial isolates

Agricultural soil irrigated with industrial wastewater (more than two decades) analysed for heavy metals revealed high levels of Fe, Cr, Cu, Zn, Ni and Cd. Out of a total of 40 bacterial isolates obtained from these soils, 17 belonged to the family enterobacteriaceae and 10 were *Pseudomonas* spp. A maximum MIC of 200 for Cd, 400 for Zn and Cu, 800 for Ni, and 1600 g/ml for Pb was observed. Biosorption of Ni and Cd studies over a range of metal ion concentrations with Escherichia coli WS11 both in single and bi-metal systems showed that the adsorption of Cd and Ni was dependent on the concentrations and followed the Freundlich adsorption isotherm. The biosorption of Ni increased from 6.96 to 55.31mg/g of cells, and Cd from 4.96 to 45.37mg/g of cells at a concentration ranging from 50 to 400 g/ml after2 h of incubation in a single metal solution. A further increase in incubation time had no significant effect on the biosorption of metals [25].

Aspergillus niger:

In this study, it was considered that the biosorption of heavy metals by biomass might occur during the bioleaching of fly ash. This work is focused on the biosorption behaviour of Al, Fe, Pb and Zn by *Aspergillus niger* during the bioleaching process. The fungal biomass was contacted with heavy metals solution which extracted from fly ash by using gluconic acid as leaching agent. The equilibrium time for biosorption was about 120 min. The biosorption experiment data at initial pH 6.5 was used to fit the biosorption kinetics and isotherm models. The results indicated that the biosorption of Al, Fe and Zn by *A. niger* biomass were well described by the pseudo-first order kinetic model.

The pseudo-second order kinetic model was more suitable for that of Pb. The Langmuir isotherm model could well describe the biosorption of Fe, Pb and Zn while the Freundlich model could well describe the biosorption of Al. Furthermore, the biosorption of metal ions decreased evidently in the presence of fly ash as compared to that in the absence of fly ash. This research showed that although the biomass sorption occurred during the bioleaching process, it did not inhibit the removal of Al, Fe, Pb and Zn evidently from fly ash [26, 38].

Microbial and plant derived biomass

Discharge of heavy metals from metal processing industries is known to have adverse effects on the environment. Conventional treatment technologies for removal of heavy metals from aqueous solution are not economical and generate huge quantity of toxic chemical sludge. Biosorption of heavy metals by metabolically inactive non-living biomass of microbial or plant origin is an innovative and alternative technology for removal of these pollutants from aqueous solution. Due to unique chemical composition biomass sequesters metal ions by forming metal complexes from solution and obviates the necessity to maintain special growth-supporting conditions. Biomass of *Aspergillus niger*, *Penicilliumchrysogenum, Rhizopus nigricans, Ascophyllum nodosum, Sargassum natans, Chlorella fusca, Oscillatoriaanguistissima, Bacillus firmus* and *Streptomyces* sp. have highest metal adsorption capacities ranging from 5 to 641 mg/ g mainly forPb, Zn, Cd, Cr, Cu and Ni. Biomass generated as a by-product of fermentative processes offers great potential for adopting an economical metal-recovery system. The purpose of this paper is to review the available information on various attributes of utilization of microbial and plant derived biomass and explores the possibility of exploiting them for heavy metal remediation [27].

Plant Root Tissues

Root tissues of two common weeds, *Amaranthusspinosus* and *Solanum nigrum*, were found to adsorb dissolved Cu2+ in aqueous solutions. The adsorption can be represented by Langmuir and Freundlich isotherms. The equilibrium adsorption level was determined to be a function of initial solution pH and temperature. The adsorption capacity decreased with decreasing pH and increasing temperature. Alkaline pre-treatment of the root tissue doubled the adsorption capacity. Ground powders of the root tissues were immobilized within alginate gel beads (3 mm diameter) for use in a packed-bed column.

The mass transfer coefficient was calculated using the rate of metal ion adsorption to the gel beads. Surface film mass transfer resistance was important. Continuous adsorption/desorption cycles for removing and concentrating Cu2+ in solution were performed using the packed-bed column, which would be useful for treating wastewater containing trace amounts of copper ions[28].

Candida lipolytica and dewatered sewage sludge

In this study, the objective was to investigate Cr removal from aqueous solutions, as well as Cr, Cu, Ni and Zn from electroplating wastewaters by the mixture of *Candida lipolytica* and sewage sludge. The bioreduction ratios of Cr (VI) and the removal ratios of total Cr showed that initial pH, biosorbent dosage and contact time were the important parameters for Cr biosorption. The range of optimal pH for the mixture (1– 5) was wider than *C. lipolytica* (1–4) and sewage sludge (2–4), respectively. Biosorption and bioreduction potentials of living *C. lipolytica* were better than those of cell wall and cytoplasm. Bonded hydroxyl group, CH2 asymmetric stretch, amide I, amide II, amide III, secondary amide, pyridine(I)b(C–H) and pyridine(II)b(C–H) were detected in the biosorbent and they were the functional groups for binding Cr. The effect of Cu and Zn in combination was significant on the removal of total Cr and the bioreduction of Cr(VI) [29].

Cyanobacterial Mats

The pH-dependent metal sorption by *Oscillatoria*and *Phormidium* dominated mats was effectively expressed by the Hill function. The estimated Hill functions can fruitfully predict the amount of metal sorbed at a particular initial pH. Pretreatment of biomass with 0.1 mmol L^{-1} HCl was more effective than pre-treatment with $CaCl_2$, HNO_3, NaOH, and SDS in enhancing metal sorption ability of the biomass. Desorption of metal ions in the presence of 100 mM L^{-1} HCl from metal-loaded mat biomass was completed within 1 h. After six cycles of metal sorption/desorption, sorption decreased by 6–15%. Only 6% and 11% of the biomass derived from the *Oscillatoria* sp. and *Phormidium* sp.dominated mats was lost during the cycling. The cyanobacterial mats seem to have better potential than several biomass types for use in metal sorption from wastewaters as they are ubiquitous, self-immobilized and have good reusability [30, 37].

Myriophyllum spicatum

Batch experiments were conducted to assess the effects of Cu (II) and Zn (II) on the biosorption of Pb (II) ions by fresh tissues of *Myriophyllum spicatum*. The biosorption of Pb (II) was examined for single, binary and ternary solutions at different initial concentrations and different pH values. The experimental results showed that the biosorption capacity increased with increasing pH from 2.0 to 6.0. Both Cu (II) and Zn (II) ions were found to have an adverse effect on the biosorption of Pb (II). The biosorption equilibrium data for single-metal solution were fitted to three isotherm models: Langmuir, Freundlich and Sips, and the Sips isotherm model gave the best fit for the experimental data. The maximum biosorption of Pb(II) in Pb–Cu binary system decreased with increasing concentration of copper ions, and the biosorption equilibrium data for the binary metal solution fitted the Langmuir competitive model well. Comparison between biosorption of Pb (II) and Cu (II) by M. spicatum in the binary solution could lead to the conclusion that the biosorbent (M. spicatum) has no preference of Pb (II) over Cu (II). Fourier transform

infrared (FT-IR) spectroscopy was used to characterize the interaction between *M. spicatum* and Pb (II) ions. The results revealed that the carboxyl, hydroxyl and carbonyl groups are the main binding sites for Pb (II) [31, 36].

Aquatic Macrophyte - Hydrilla verticillata

The removal of heavy metal ions by the nonliving biomass of aquatic macrophytes was studied.We investigated Cd biosorption by dry *Hydrilla verticillata* biomass. Data obtained in batch experiments indicate that *H. verticillata* is an excellent biosorbent for Cd. Cd was rapidly adsorbed and such adsorption reached equilibrium within 20 min. The initial pH of the solution affected Cd sorption efficiency. Results obtained from the other batch experiments conformed well to those obtained using the Langmuir model. The maximum adsorption capacity qmax for *H. verticillata* was 15.0 mg/g for Cd. The breakthrough curve from the continuous flow studies shows that H. verticillata in the fixed-bed column is capable of decreasing Cd concentration from 10 to a value below the detection limit of 0.02 mg/l. The presence of Zn ions affected Cd biosorption. It can be concluded that *H. verticillata* is a good biosorbent for treating wastewater with a low concentration of Cd contaminants [7, 39].

Algal bioremediation - Spirogyra and Cladophora filamentous macroalgae

The aim of this research was to develop a low cost adsorbent for wastewater treatment. The prime objective of this study was to search for suitable freshwater filamentous algae that have a high heavy metal ion removal capability. This study evaluated the biosorption capacity from aqueous solutions of the green algae species, *Spirogyra* and *Cladophora* for lead (Pb (II)) and copper (Cu (II)). In comparing the analysis of the Langmuir and Freundlich isotherm models, the adsorption of Pb (II) and Cu (II) by these two types of biosorbents showed a better fit with the Langmuir isotherm model. In the adsorption of heavy metal ions by these two types of biosorbents, chemical and physical adsorption of particle surfaces was perhaps more significant than diffusion and adsorption between particles. Continuous adsorption–desorption experiments discovered that both types of biomass were excellent biosorbents with potential for further development [12, 40].

The chemical process

Orange peel

The use of orange peel (OP) as a biosorbent material presents strong potential due to its high content of cellulose, pectin (galacturonic acid), hemicellulose and lignin. As a low cost, orange peel is an attractive and inexpensive option for the biosorption removal of dissolved metals. Equilibrium, thermodynamic and kinetic studies were carried out for the biosorption of $Pb2^+$, $Cd2^+$ and $Ni2^+$ ions from aqueous solution using the grafted copolymerization-modified orange peel (OPAA). Langmuir and Freundlich isotherm models were applied to describe the biosorption of the metal ions onto OPAA. The influences of pH and contact time of solution on the biosorption were studied. Langmuir model fitted the equilibrium data better than the Freundlich isotherm. According to the Langmuir equation, the maximum uptake capacities for $Pb2^+$, $Cd2^+$ and $Ni2^+$ ions were 476.1, 293.3 and 162.6mgg^{-1}, respectively. Compared with the unmodified orange peel, the biosorption capacity of the modified biomass increased by 4.2, 4.6 and 16.5fold for $Pb2^+$, $Cd2^+$ and Ni2+, respectively. The kinetics for $Pb2^+$, $Cd2^+$ and $Ni2^+$ ions biosorption followed the pseudo-second-order kinetics. The free energy changes for $Pb2^+$, $Cd2^+$ and $Ni2^+$ ions biosorption process were found to be 3.77, −4.99 and −4.22 kJ mol^{-1}, respectively, which indicates the spontaneous nature of biosorption process. FTIR demonstrated that carboxyl and hydroxyl groups were involved in the biosorption of the metal ions. Desorption of $Pb2^+$, $Cd2^+$ and $Ni2^+$ ions from the biosorbent was effectively achieved in a 0.05 mol L^{-1} HCl solution [2, 41].

Sugar-beet pulp pectin gels

The present work reports the feasibility of using sugar-beet pectin gels for the removal of heavy metals from aqueous solutions. Sugar-beet pectin hydro- and xerogels were tested in the batch biosorption and desorption of cadmium, lead and copper. Pectins were successfully extracted and demethylated from the sugar-beet pulp, an agricultural residue, and gelled in the presence of CaCl2. The stability of the hydro and xerogel pectin beads made them suitable for biosorption of heavy metals in different conditions. Biosorption data were fitted to the pseudo-second order kinetic model and the Langmuir isotherm model, obtaining the corresponding parameters. Treated and untreated beads were characterized using FTIR and SEM to determine possible binding mechanisms. The main mechanisms involved were ion exchange with calcium of gel structure and chelation or complexation with carboxyl groups. After biosorption, calcium in the gels was substituted by metal cations reorganizing the structure of the gel matrix in a way that was visible using scanning electron microscopy. HNO_3 0.1M was the best eluant for the reutilization of the gels and recovered all the adsorbed metal unlike HCl and H_2SO_4. Sugar-beet pectins could be used as an efficient biosorbent for the treatment and recovery of Cu, Pb and Cd from wastewater [6].

Activated sludge

Activated sludge was collected as slurry from the thickener of the wastewater treatment facility in a dairy factory. Biosorption experiments were performed using different pre-treated activated sludge as bioadsorbent for $Cu2^+$, $Cd2^+$ and $Ni2^+$. Pretreatment with NaOH was found to improve the adsorption capacity of the sludge where as treatment with HCl reduces it. The treated and untreated sludge show great affinity towards $Cu2^+$, $Cd2^+$ and low affinity towards $Ni2^+$. The maximum loading capacity of the NaOH treated sludge was 131.6 and 93.4 mg/g for Cu^{2+} and Cd^{2+}, respectively. It was found that

both Langmuir and Freundlich isotherm models fit the adsorption data with the different types of sludge and ions. The value of R2 exceeds 0.95 in most cases. It was found that the maximum adsorption capacity drops 18.3% as the temperature increases from 25 to 45ºC. The adsorption capacity was found to increase as the adsorbent mass and mixing speed increase. The optimum pH corresponding corresponds to maximum adsorption efficiency was found to be about 5. Desorption experiments indicate that the desorption efficiency with 0.1 M H_2SO_4 solution reaches 94% in the first cycle and decreases to 8% in the 4th cycle after collecting 20,700 mg $Cd2^+$/l H_2SO_4 [8].

Tobacco dust

A typical lignocellulosic agricultural residue, namely tobacco dust, was investigated for its heavy metal binding efficiency. The tobacco dust exhibited a strong capacity for heavy metals, such as Pb (II), Cu (II), Cd (II), Zn (II) and Ni (II), with respective equilibrium loadings of 39.6, 36.0, 29.6, 25.1 and 24.5 mg of metal per g of sorbent. Moreover, the heavy metals loaded onto the biosorbent could be released easily with a dilute HCl solution. Zeta potential and surface acidity measurements showed that the tobacco dust was negatively charged over a wide pH range (pH > 2), with a strong surface acidity and a high OH adsorption capacity. Changes in the surface morphology of the tobacco dust as visualized by atomic force microscopy suggested that the sorption of heavy metal ions on the tobacco could be associated with changes in the surface properties of the dust particles. These surface changes appeared to have resulted from a loss of some of the structures on the surface of the particles, owing to leaching in the acid metal ion solution. However, Fourier transform infrared spectroscopy (FTIR) showed no substantial change in the chemical structure of the tobacco dust subjected to biosorption. The heavy metaluptake by the tobacco dust may be interpreted as metal–H ion exchange or metal ion surface complexation adsorption or both [9, 42].

Peanut shell

Biosorption of Cu (II) and Cr (III) ions from aqueous solutions by peanut shell biomass were investigated as a function of initial pH, initial biomass concentration and temperature. The optimum sorption conditions were studied for each metal separately. The kinetics and equilibrium of biosorption were examined in detail. Four kinetic models (pseudo-first order, pseudo-second order, power function equation, and Elovich model) were used to correlate the experimental data and to determine the kinetic parameters. Four well-known adsorption isotherms were chosen to describe the biosorption equilibrium. The experimental data wereanalyzed using two two-parameter models (Langmuir and Freundlich) and two three-parameter models (Redlich–Peterson and Sips). The equilibrium biosorption isotherms showed that peanut shells possess high affinity and sorption capacity for Cu(II) and Cr(III) ions, with monolayer sorption capacities of 25.39 mg $Cu2^+$and 27.86 mg $Cr3^+$ per 1 g biomass, respectively. All results showed that peanut shells biomass is an attractive, alternative low-cost biosorbent for removal of heavy metal ions from aqueous media [10, 43].

Polypeptides

MerP protein possesses a highly conserved domain with two cysteine residues for $Hg2^+$ binding. The MerP protein originated from mer operon of Gram-positive bacterium Bacillus cereus RC607 possesses adjoining two cysteines while that of Gram-negative bacterium *Pseudomonas* sp. K-62 contains two cysteines separated by spacing two other residues. Both heterogeneous merP genes were cloned and over-expressed in Escherichia coli BL21 hosts. The resulting recombinant strains exhibited a six- to eightfold increase in $Hg2^+$ resistance and a 10% increase in $Hg2^+$ adsorption capacity. The merP over-expressed strain harbouring Gram-positive merPhad 142, 84 and 33% increase for $Cu2^+$, $Cd2^+$ and $Pb2^+$ adsorption capability, respectively, over that of merP-free host cells. The strain carrying Gram-negative merP also increased 47, 55 and 12% for $Cu2^+$, $Cd2^+$ and $Pb2^+$ adsorption, respectively. Multi-metal biosorption experiments showed that the affinity of metal biosorption by the recombinant strains decreased in the order of Cu > Pb > Cd. Peptides containing the amino acid sequences of metal-binding motif for both heterogeneous MerP proteins were chemically synthesized and covalently immobilized on Celite carriers to examine their metal-binding ability. For Bacillus MerP-originated peptides, the binding capacity was 0.72, 0.45 and 0.36 mol/mol peptide for Cu, Cd and Pb, respectively, while the capacity was 0.51 0.45 and 0.31 mol/mol peptide for Cu, Cd and Pb, respectively, for the peptide containing *Pseudomonas* MerP metal-binding motif.These results may show that MerP with adjoining cysteines seems to be more effective in binding metals than that with two separated Cysteines [11].

Up flow sludge columns

The present study was carried out for evaluating the retention behaviour of sanitary sewage and sand in relation to chromium and nickel ions in up flow reactors. It was found that the sludge presented a greater assimilation of the metals studied when compared tothe inert material, probably due to the presence of anionic groups, which favours adsorption and complexation processes. Thermal analyses of the samples showed a shift in the decomposition peaks of the ‘‘in natura’’ sludge, when compared with those of the samples spiked with the metals, confirming the possibility of interactions between the heavy metals and the anionic groups present in the sludge [19].

Green coconut shell powder

The effective removal of heavy metals from aqueous wastes is among the most important issues for many industrialized countries. The traditional treatment methods used to remove heavy metals from wastewaters have certain disadvantages such as incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require disposal. The search for alternative and innovate

treatment techniques has focused attention on the use of biological materials for metal removal and recovery technologies. Biosorption has gained important credibility during recent years because of its good performance and low cost [33].

In the present study, the biosorption capacity of powder from coconut shell was studied for cadmium. The adsorption capacity of biomass was investigated by batch experiments. The influence of metal ion concentration and pH were evaluated and the results were fitted using adsorption isotherm mode(ls. The kinetic of cadmium biosorption was also investigated [22].

Activated sludge

The biosorption of different metals ($Cu2^+$, $Cd2^+$, $Zn2^+$, $Ni2^+$ and $Pb2^+$) was investigated using activated sludge. The optimum pH was 4 for Cd, Cu and Pb sorption and 5 for Ni and Zn. Biomass metal uptake clearly competed with protons present in the aqueous medium, making pH an important variable in the process. Protons consumed by biomass in control tests versus protons exchange in biosorption tests confirmed a maximum exchange between metal cations and protons at pH 2. The study of the influence of biomass concentration revealed that the amount of protons released from biomass increased with biomass concentration. This would confirm the hypothesis of ion exchange between both types of ions.
The application of the Langmuir and Freundlich models showed a better fitting of experimental data to the first model. The maximum sorption uptake of the studied metals by the activated sludge showed the following decreasing order: Pb4>Cu4>Cd4>Zn4>Ni. Desorption experiments showed that HCl was a good eluent for the five metals tested, particularly at low pH values (1 and 2). At pH 3or 4 the desorption yield was significantly lower. However, its use did not allow the reuse of biomass in subsequent loading and unloading cycles. EDTA was also a good desorption agent, achieving the total recovery for the five metals tested at a concentration of 1mM, with the advantage that biomass could be reused for three sorption–desorption cycles [24].

Short hemp fibres

Sorption potential of waste short hemp fibres for Pb2+, Cd2+ and Zn2+ ions from aqueous media was explored. In order to assess the influence of hemp fibre chemical composition on their heavy metals sorption potential, lignin and hemicelluloses were removed selectively by chemical modification. The degree of fibre swelling and water retention value were determined in order to evaluate the change in accessibility of the cell wall components to aqueous solutions due to the fibre modification. The effects of initial ion concentration, contact time and cosorption were studied in batch sorption experiments. The obtained results show that when the content of either lignin or hemicelluloses is progressively reduced by chemical treatment, the sorption properties of hemp fibres are improved. Short hemp fibres are capable of sorbing metal ions (Pb2+, $Cd2^+$ and $Zn2^+$) from single as well as from ternary metal ion solutions. The maximum total uptake capacities for $Pb2^+$, $Cd2^+$ and $Zn2^+$ ions from single solutions are the same, i.e.0.078 mM/g, and from ternary mixture 0.074, 0.035 and 0.035 mM/g, respectively [32].

CONCLUSION

Bioremediation is still considered to be a developing technology. One difficulty is that bioremediation is carried out in the natural environment, which contains diverse uncharacterized organisms. Most pollutant-degrading microorganisms isolated and characterized in the laboratory are now thought to make a minor contribution to bioremediation. Another difficulty is that no two environmental problems occur under completely identical conditions; for example, variations occur in the types and amounts of pollutants, climate conditions and hydrogeodynamics. These difficulties have caused the bioremediation field to lag behind knowledge-based technologies that are governed by common rationales. As summarized in this review, information on microbial populations relevant to bioremediation is accumulating rapidly with the aid of molecular ecological approaches. Although our knowledge is not yet complete, it is time to initiate more comprehensive approaches to find common rationales in bioremediation. In some cases, for example, marine petroleum bioremediation, we have already found that similar bacterial populations occur even at geographically distant sites. Understanding the physiology and genetics of such populations may prove very useful to assess and improve bioremediation. Most importantly, we need to identify general aspects in certain types of bioremediation. For this purpose, I wish to propose the construction of a database that collects the results of molecular ecological assessments of contaminated and bioremediated sites. The database would provide bioremediation with ecological backgrounds and, in concert with currently available databases relevant to bioremediation, would facilitate the development of commonly applicable schemes for certain types of bioremediation [34, 35].

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